STIC-ILL

1627 8AM2 09/1,352

From: Garcia, Maurie
Sent: Tuesday, May 23, 2000 7:09 PM
To: STIC-ILL
Subject: RE: Reference request

NO 5/24

Please send me a copy of the following reference ASAP.
PLEASE NOTE: My mailbox is located at Crystal Mall 1 2E12. Please deliver references there.
THANKS!!

ACCESSION NUMBER: 1995:231833 CAPLUS
DOCUMENT NUMBER: 122:23112
TITLE: The role of microplate selection and assay design in the application of automation and robotics
AUTHOR(S): Kolb, Alfred J.
CORPORATE SOURCE: Packard Instrument Company, 800 Research Parkway, Meriden, CT, 06450, USA
SOURCE: Chemom. Intell. Lab. Syst. (1994), 26(2), 107-13
CODEN: CILSEN; ISSN: 0169-7439
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Advances in the automation of high-throughput screening for drug discovery are being made in three interrelated areas. These three areas are instrumentation, robotics and assay technol. Liq. handling systems that are designed for a variety of sample container sizes, esp. the widely used 96-well microplate, were the first instruments to be integrated into robotics systems. However, anal. instruments have not been widely linked to robots. This is primarily because of the complexity of prepg. assays for anal. Transferring samples to vials or tubes for anal., while tech. possible, was not suited to the anal. of thousands of samples a day. The

Wall 5/25

L.C 5/25 NOBOWN RC

VOL. 26, NO. 1 CHEMOMETRICS AND INTELLIGENT LABORATORY SYSTEMS: LABORATORY INFORMATION MANAGEMENT MAY 1994

Abstracts / Contents Lists published in Analytical Abstracts, ASCA, BioSciences Information Service, Chemical Abstracts, Chromatography Abstracts, Current Contents / Engineering, Technology & Applied Sciences, Current Contents / Physical, Chemical & Earth Sciences, Current Index to Statistics (core journal), Excerpta Medica, INSPEC, SCISEARCH, and Cambridge Scientific Abstracts

CONTENTS

TUTORIAL

ORIGINAL RESEARCH PAPERS

CASE STUDY

MONITOR

0925-5281 / 94 / $07.00

ELSEVIER

Laboratory Information Management 26 (1994) 107–113

Chemometrics and intelligent laboratory systems: Laboratory information management

The role of microplate selection and assay design in the application of automation and robotics

Alfred J. Kolb

Packard Instrument Company, 800 Research Parkway, Meriden, CT 06450, USA

Received 6 April 1994; accepted 19 June 1994

Abstract

Advances in the automation of high-throughput screening for drug discovery are being made in three interrelated areas. These three areas are instrumentation, robotics and assay technology. Liquid handling systems that are designed for a variety of sample container sizes, especially the widely used 96-well microplate, were the first instruments to be integrated into robotics systems. However, analytical instruments have not been widely linked to robots. This is primarily because of the complexity of preparing assays for analysis. Transferring samples to vials or tubes for analysis, while technically possible, was not suited to the analysis of thousands of samples a day. The introduction of microplate counters, such as the multidetector TopCount Microplate Scintillation and Luminescence Counter, allows both the assay and the analysis to take place in the microplate. This has changed microplate and assay design so that automation is a relatively simple robotics exercise. It is the recent developments in assay technology that are making researchers take a new look at robotics. New assays are being developed that eliminate the complex separation steps often required in biological assays. Referred to as homogeneous or non-separation, these assays are easily automated and are ideal for high-throughput screening. Homogeneous assay formats have been developed for radioisotopic assays (scintillation proximity assay and FlashPlates) as well as for luminescence assays for cell proliferation, chemical toxicity and multidrug resistance (CytoLite). The design of new homogeneous assays and microplates that simplify separation steps are leading to increased interest in automation and robotics.

1. Introduction

Discussions of automation most frequently focus on the suitability and cost of instruments and robotics. However, when discussing microplate-based biological assays, the design of the assay and the selection of the appropriate microplate are key factors. This paper will focus on how the assays, microplates, reagents and instruments can influence automation and robotics.

2. The role of microplates in instrument and assay development

The popularity of the microplate as a standard for biological assays has significantly influenced the development of automation and robotics. The effect on instrumentation is the most obvious. The standard format of the microplate has led to the development of everything from multichannel hand-held pipettes to automated liquid handling systems, analytical instruments, and complete robotic systems. However, there have been dramatic advances in assay developments that are less recognized, but have a significant impact on automation.

Until recently, the analysis of radioactive and luminescent samples was done in individual scintillation vials or test tubes. Even if the assays were incubated in microplates, the samples had to be processed to separate bound from free label before being transferred to vials

SSDI 0925-5281(94)00006-G

for analysis. Separation steps are a very tedious and time-consuming process that often includes pipetting, organic extractions, filtration, precipitation or centrifugation. Many advantages of microplates such as small sample volumes, high sample density, standard format for automation and ease and safety of handling were lost because of the transfer steps.

The transfer of samples from microplates to scintillation vials effectively isolated the assay into three distinct phases: preparation, separation and analysis. Any microplate could be used for assay preparation and incubation since it simply acted as an inert vessel. However, the development of microplate counters such as the TopCount Microplate Scintillation and Luminescence Counter (Packard Instrument Company) and microplate luminometers (Dynatech Laboratories) dramatically changed the role of the microplate. Both the incubation and analysis of the assay can now be done in the same microplate. The use of the plate as an active component of the assay has become a powerful new tool in assay design.

3. Assays differ in the complexity of processing steps

Assays can be divided into several broad categories that are distinguished by the number and complexity of the steps involved. This in turn defines the level of complexity to achieve automation. The most common assays, illustrated in Fig. 1, can be divided into homogeneous, in-plate, and filtration.

Homogeneous assays are typically radioisotopic or luminescent. Fig. 1a demonstrates a homogeneous assay using the example of a luminescent probe for measuring cell viability. The bound luminescent probe is activated to produce light by the interaction with reactive oxygen species produced within living cells. The free probe or the probe attached to dead cells does not participate in the reaction and does not produce light. This eliminates the need to separate the unbound probe. Homogeneous assays are the simplest to automate since only assay preparation, incubation and analysis are required. However, they are also the most difficult to design and the necessary reagents are not always available. There is a significant amount of research into the development of new homogeneous assay formats.

In-plate assays can be radioactive, luminescent, colorimetric or fluorescent. Fig. 1b is a typical radioactive in-plate assay. The radioactivity to be measured (ligand, antigen, second antibody) is bound to the wall of the microplate through an attached receptor or antibody. The free radioactivity would interfere with the measurement of the bound activity, so it must be removed by aspiration. The radioisotope bound to the well of the plate is easily quantitated after the addition of scintillation cocktail. While in-plate assays are not as simple as homogeneous assays, they do not require complex processing. There are many assays that can be converted to in-plate assays and methods for attaching biological macromolecules to polystyrene plates have been published [1,2].

Fig. 1c represents an assay that requires a filtration step to separate bound from free label. This is the most common method of performing receptor binding assays. The receptor, radioligand, and other necessary components are incubated in solution. The entire mixture is harvested through a filter which retains the receptor-bound radioligand but allows the unbound ligand to pass through. While it is one of the most difficult procedures to automate, it is also a popular and widely used method.

4. Homogeneous assays

Homogeneous assays are designed to eliminate all separation steps. The reagents are pipetted, incubated, and analyzed in the same microplate with no separation, harvesting or transfer requirements. This category of assay is in great demand when large numbers of samples must be processed. The CytoLite test (Packard Instrument Company) and the Scintillation Proximity Assay (SPA, Amersham Corporation) are two assays that have been specifically designed as homogeneous.

CytoLite is a luminescence test for measuring cell proliferation or cell toxicity. After the cells are grown under the appropriate experimental conditions, a reagent is pipetted directly into the culture media and the long-lived luminescence is measured. There is no need for harvesting, incubation, or solubilization procedures. Cell proliferation assays are also done using ^{3}H-thymidine uptake into DNA or by colorimetric assays. Fig. 2 illustrates the measurement of cell proliferation

Fig. 1
measu
intera
are re
more

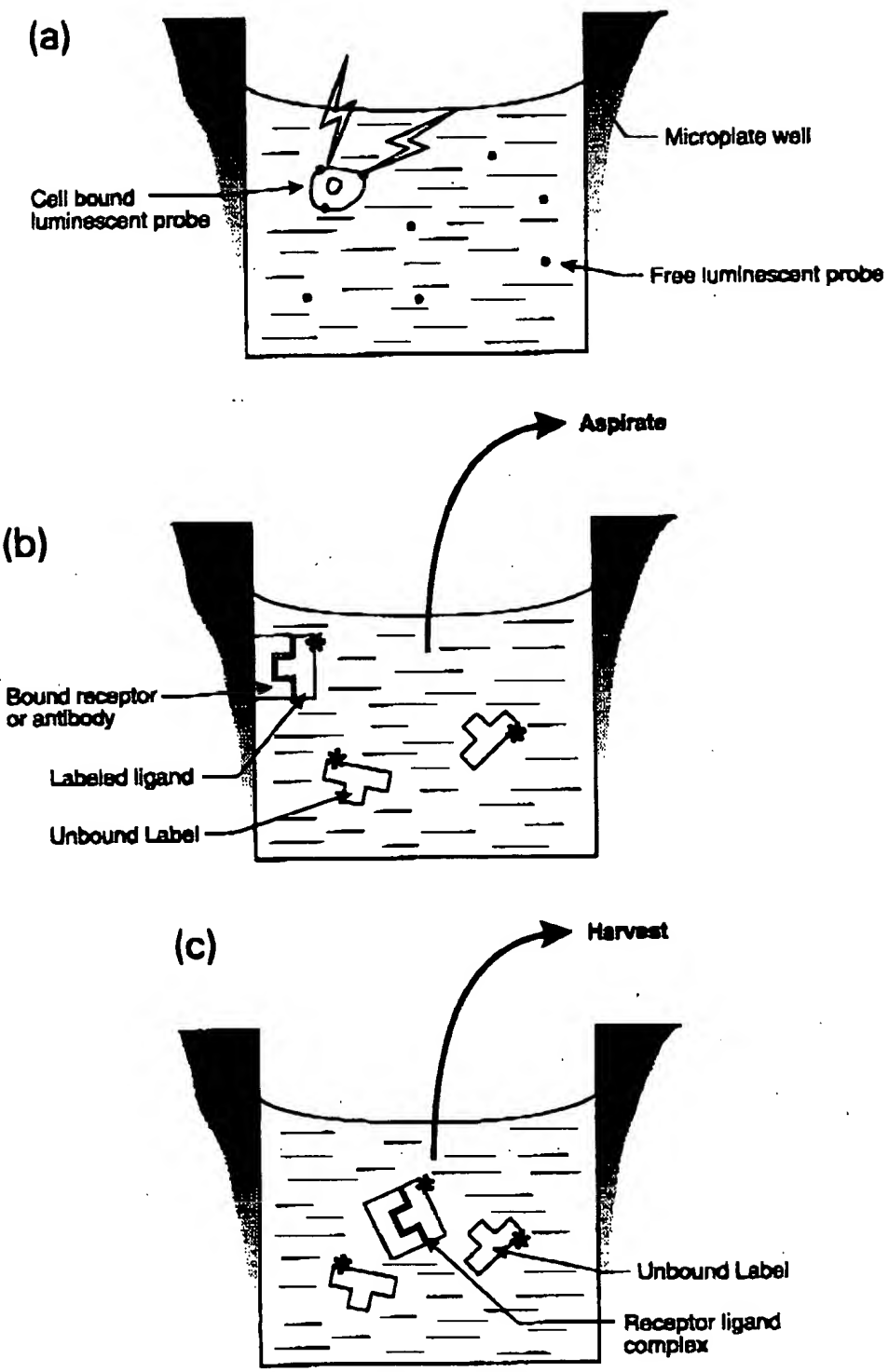

Fig. 1. Homogeneous, in-plate and filtration assays require different processing steps. (a) Homogeneous assays are prepared, incubated and measured in the same microplate without any processing steps. These are the simplest assays to automate. (b) In-plate assays use biological interactions to bind the label to be measured to the plate. The assays are prepared and measured in the same plate, but an aspirate and wash step are required to remove unbound label. (c) Separation assays require filtration, or other methods, to separate bound from free label. This requires more processing than the methods illustrated in panels (a) and (b).

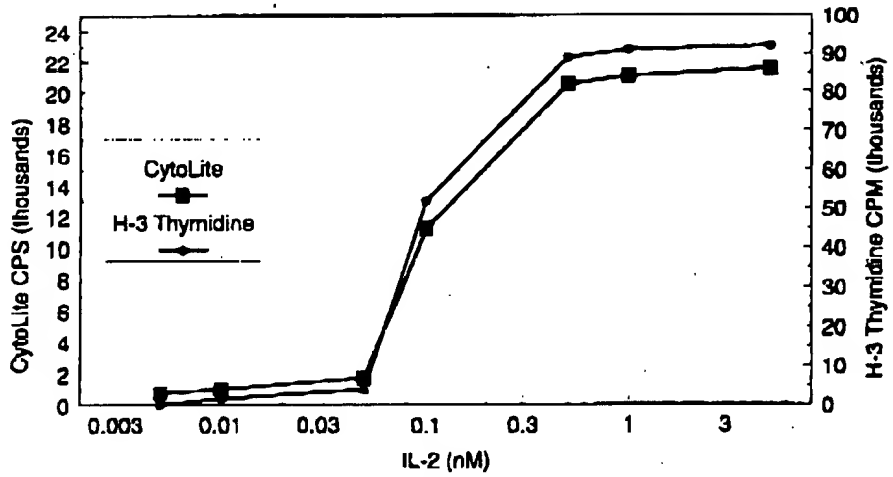

Fig. 2. A comparison of a homogeneous luminescence assay with a radioactive filtration assay for cell proliferation. CTL-4 (IL-2 dependent T-lymphocytes) cells were incubated in the presence of recombinant, human interleukin-2. Cell proliferation was measured by luminescence (CytoLite) counts per second or ^{3}H-thymidine counts per minute as described [3].

with the homogeneous luminescence assay compared to the traditional ^{3}H-thymidine uptake [3].

SPA is an assay technique using the radioisotopes ^{3}H or ^{125}I. Scintillating beads are used that produce light when they absorb the energy of a radioactive decay [4]. The assays are designed so that the radioactive label of interest is bound to the bead through the appropriate biological interaction. This places the label in close proximity to the bead so it interacts and produces light. The unbound or free label is not measured because the decay energy is absorbed by the aqueous media before it can interact with a bead. This eliminates the need for any separation steps.

FlashPlates are a recent development that combine the chemistry of polystyrene with a coating of scintillating material. As with a standard in-plate assay, receptors [5] and antibodies can be bound to the scintillating layer on the wells of the plate. The radiolabeled compound to be measured is then bound to the receptor. Since the wells are coated with a thin layer of plastic scintillator, the addition of liquid scintillation cocktail is eliminated. Fig. 3 illustrates the concept of FlashPlate assays. The radiolabeled molecule bound to the well interacts with the scintillator to produce light. The steps of harvesting, washing and cocktail addition are eliminated.

Homogeneous assays are the easiest to automate. A liquid handling system such as the MultiPROBE are widely available and can be used to automatically dispense reagents while the plates can be manually trans-

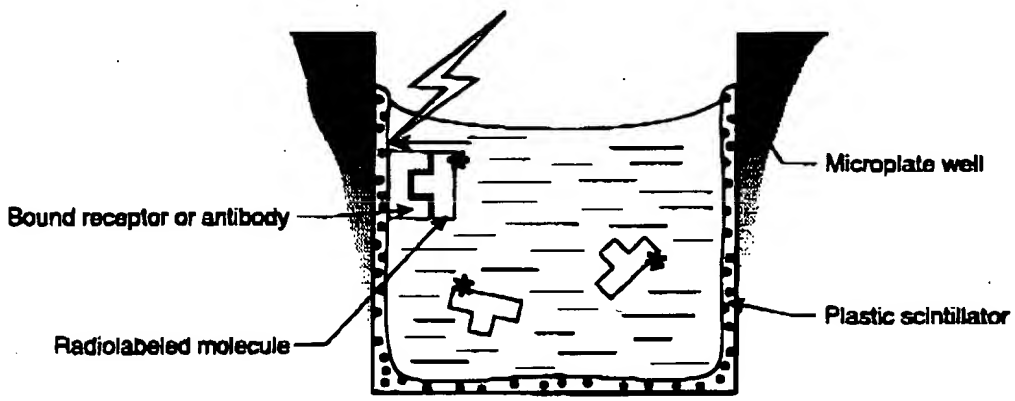

Fig. 3. Diagrammatic explanation of a microplate to simplify assay processing. Microplates are being developed to meet specific assay requirements. This illustrates a microplate with a thin layer of plastic scintillator (FlashPlate) on the inside of the wells. Combining the advantages of homogeneous and in-plate assays reduces sample processing.

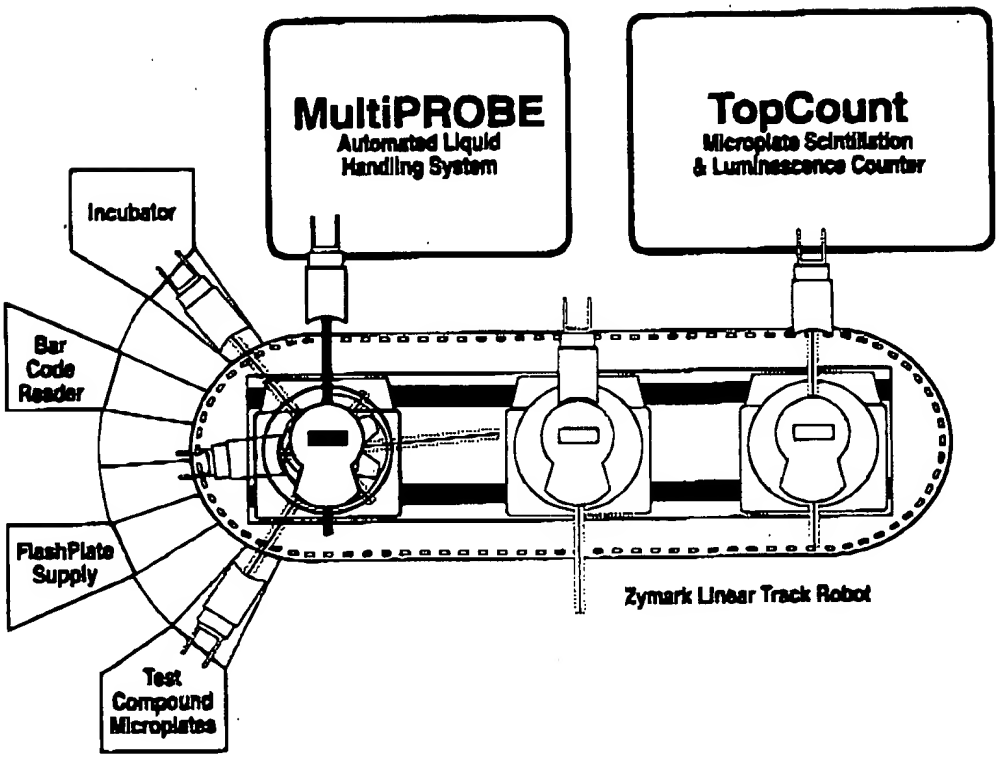

Fig. 4. Commercially available instruments and robots can automate a range of homogeneous and in-plate assays. A Zymark robot has been used to link together liquid handling and measurement instruments to meet a variety of laboratory requirements. Instrument and robot companies are working together to meet more demanding research applications.

ferred for incubation and analysis. Alternatively, the entire process can be automated with a robotic arm. Fig. 4 is a robotic system developed by Zymark for homogeneous assays. The robot, liquid handler, analytical instrument and associated equipment are commercially available. The ability to add equipment and modify assay protocols makes these systems flexible enough to meet the changing requirements of high-throughput laboratories.

5. In-plate assays

In-plate assays take advantage of the well known chemistry of polystyrene to specifically bind biological molecules. Assays using adherent cells, protein A coating for immunoassays and streptavidin–biotin interactions are common examples of in-plate assays. The labeled molecule of interest (radioactive, luminescent) is bound to the well of the plate through specific biological interactions. The unbound label is removed by a simple wash step.

These assays can be automated with the same instruments used for homogeneous assays. The aspirate and wash steps are efficiently carried out with standard microplate washers.

6. Filtration assays

Assays such as membrane receptor binding, ^{3}H-thymidine uptake for cell proliferation, and precipitate collection require harvesting samples onto filters as a way to separate bound from free label [6]. Harvesting can be a time-consuming, labor-intensive and error-prone process. This makes it an ideal candidate for automation. The automation of filtration assays has been very limited because of the difficulty in robotic handling of glass fiber filters, the most commonly used filter in receptor and cell harvesting. Harvesters (Brandel, Skatron) are available that somewhat automate the process by harvesting 96 samples simultaneously. However, the operator must stay in front of the harvester to initiate harvesting and to replace the filters and assay plates.

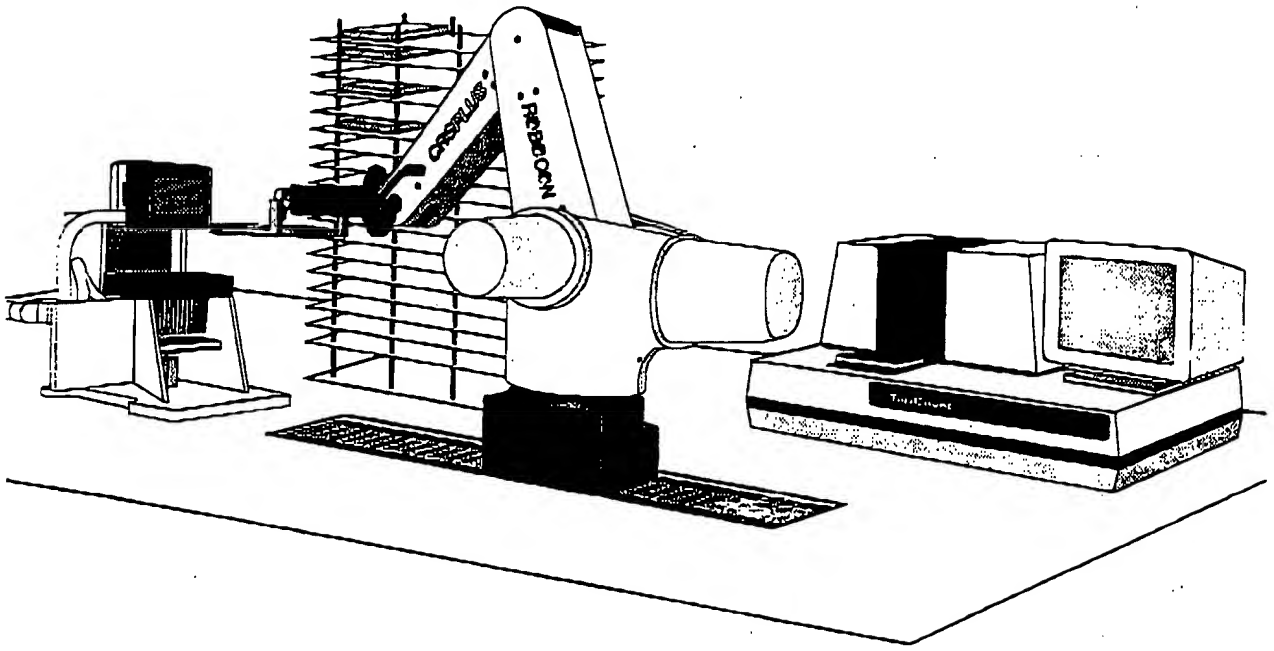

Fig. 5. Filtration assays could be automated with the development of microfiltration plates. The automation of filtration assays was first accomplished by Robocon using a CRS robot. This was made possible with the development of UniFilter, a microplate with an integral glass fiber filter. Liquid handling, incubation and other ancillary equipment can be incorporated into open systems.

Preparing the filter sheets or individual filter disks for counting is a completely manual process.

The development of microplates containing integrated glass fiber filters (UniFilters) has allowed the complete automation of harvesting assays. While individual filters cannot be manipulated by a robot, a microplate can be easily gripped, moved and accurately positioned. The first example of the complete automation of harvesting has been developed by Robocon (Vienna, Austria). Fig. 5 shows some of the equipment that can be incorporated into this robotic system. A commercially available FilterMate harvester (Packard Instrument Company) has been modified by the addition of motors and solenoids so that it is under the complete control of the robot system controller. The robotic arm and hand precisely positions the UniFilter filtration plate on the harvester. The assay plate, after system-controlled time and temperature incubation, is then positioned under the wash/aspirate tubes of the harvester. The entire harvesting and washing procedure is automated based on the operator-defined protocol. After harvesting, the robot adds scintillant and positions the UniFilter on the TopCount for measurement.

The development of automated filtration assays is an example of how robotics can offer a complete and practical solution to a difficult and tedious procedure.

7. Automated instruments and robotics must meet laboratory requirements for assay diversity

The robot systems discussed here can be configured for a range of assays. Many laboratories will often run assays that include homogeneous, in-plate and filtration in the same time period. These assays can also change dramatically from month to month. These diverse and changing applications require a high degree of flexibility in a system.

The use of instruments specifically designed for functions such as pipetting, filtering and quantitation have proven themselves to be accurate, reliable and able to adapt to a wide range of assays. For some applications, the manual transfer of microplates for incubation, washing, harvesting and other intermediate steps is not rate-limiting in productivity. For other applications such as high throughput, 24-h operation, assay consistency, or the use of biohazardous materials, it is desirable to automate these steps. A robotic arm can be integrated as part of a complete assay system with existing, proven equipment. A bench of relatively small dimensions can be visualized containing an array of instruments and devices capable of performing many diverse assays. As needs change, new assays can be

programmed and new instruments can be added to an open architecture system.

8. Conclusion

The requirement to process more samples in less time is a continuing trend in industrial and academic laboratories. Diagnostic testing, high-throughput screening for drug discovery and the human genome project are examples of applications that require the processing of large numbers of samples. Because of demands such as these, a range of instrumentation has been developed for the automation of dedicated functions such as pipetting, harvesting and counting samples in microplates. The increased output of these instruments has created the need for more efficient assays and more sophisticated levels of automation. However, it is not just hardware that drives automation. The instruments and robotic systems used must be selected based on the assay itself. The development of ingenious assay systems (homogeneous and in-plate assays) and innovative microplates (filtration plates and scintillator coated plates) have combined with automation to give a coordinated and complete solution to many laboratory procedures.

The use of automation and robotics will continue to grow as long as productivity demands continue and the researcher has a choice of instruments and systems available that best meet the individual laboratory requirements.

References

[1] J. Nichols, H. LeVine, G. Smith, D. Wypij and J. Wiseman, Determination of endothelin by an immobilized receptor assay utilizing a 96-well format, *Journal of Biochemical and Biophysical Methods*, 25 (1992) 173–184.

[2] J.T. Wu and M. Erali, Development of radioimmunoassays on microplate: Application for CA–GI and CA–BR tumor markers, *Journal of Clinical Laboratory Analysis*, 7 (1993) 341–347.

[3] C. Roelant, Introduction to CytoLite: *A Chemiluminescent Reagent System for Cell Proliferation and Cytotoxicity Testing*, LiteGuides, LGA-001, Packard Instrument Company, 1993.

[4] S. Udenfriend, L.D. Gerber, L. Brink and S. Spector, Scintillation proximity radioimmunoassay utilizing ^{125}I-labelled ligands, *Proceedings of the National Academy of Sciences USA*, 82 (1985) 8672–8676.

[5] J.T. Holland, P. Singh, J.C. Brennand and A. Garman, A non-separation microtitre plate receptor binding assay, *Analytical Chemistry*, in press.

[6] N. Roessler, D. Englert and K. Neumann, New instruments for high throughput receptor binding assays, *Journal of Receptor Research*, 13 (1993) 135–145.